Synthese van peptiden met behulp van ethoxyethyn

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SUMMARY

Chapter I

In this chapter some of the most important known processes used in peptide syntheses are discussed briefly.

Chapter II

A simple process for the synthesis of peptides using acetylenic ethers is described. Ethoxyethyne was mostly used, as this is the most readily available acetylenic ether.

We describe the synthesis of a number of N-acyl-dipeptide-esters and higher N-acyl-peptide esters from N-acyl-amino-acids (N-acyl-peptides) and amino acid esters (peptide esters).

The syntheses were carried out in three ways, viz. according to:

METHOD A, by warming an intimate mixture of the components in the absence of a solvent, the amino component being added as its hydrochloride (or hydrobromide).

METHOD B, by working in boiling ethyl acetate and using the amino component as the hydrochloride (or hydrobromide).

METHOD C, by working with the free amino component in boiling ethyl acetate.

The overall reaction can be pictured as follows:

$$Ac\text{-}NHCHRCOOH + (HCl).H_2NCHR'COOR'' + HC{\equiv}COC_2H_5 \longrightarrow$$

$$Ac\text{-}NHCHRCO\text{-}NHCHR'COOR'' + CH_3COOC_2H_5 + (HCl)$$

The best results were obtained in boiling ethyl acetate containing 0.5% of water and by using a three to five fold excess of ethoxyethyne.

Method A was very quick but did not afford the best yields. The results are listed in tables I and II (chapter II, § 2).

Method B has mostly been used for dipeptide preparations and gave good results (see Table III, chapter II, § 2). Tripeptides were also obtained successfully but in some cases partial racemisation was observed (see table IV, chapter II, § 2).

Methode C proved to be the most suitable for higher peptides, because racemisation was then prevented (see table V, chapter II, § 2).

By means of these methods the hexapeptide pro-val-lys-val-tyr-pro (the order 19 to 24 inclusive in β-corticotropin from hogs) has been prepared.

Chapter III

This chapter deals with the mechanism of this new peptide synthesis and the problem of racemisation.

Three mechanisms are proposed. The first mechanism (scheme I) consists of a reaction of the amino acid ester III with the primary addition product II of ethoxyethyne and the N-acyl-amino acid, yielding an unstable adduct IV, which decomposes into the N-acyl-peptide ester V and ethyl acetate.

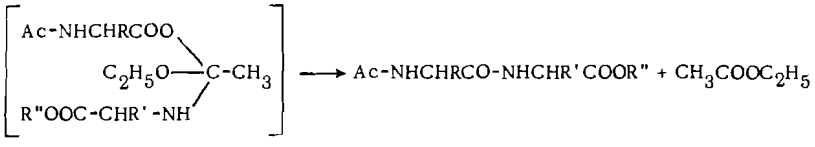
Scheme I:

$$Ac\text{-}NHCHRCOOH + HC{\equiv}COC_2H_5 \longrightarrow Ac\text{-}NHCHRCOOC(OC_2H_5){=}CH_2$$

I II

$$Ac\text{-}NHCHRCOOC(OC_2H_5){=}CH_2 + H_2NCHR'COOR'' \longrightarrow$$

II III



IV V

The second mechanism (scheme II) involves the anhydride VI of the N-acyl-amino acid.

Scheme II:

$$2\ Ac\text{-}NHCHRCOOH + HC{\equiv}COC_2H_5 \longrightarrow (Ac\text{-}NHCHRCO)_2O + CH_3COOC_2H_5$$

I VI

$$(Ac\text{-}NHCHRCO)_2O + H_2NCHR'COOR'' \longrightarrow Ac\text{-}NHCHRCO\text{-}NHCHR'COOR'' + Ac\text{-}NHCHRCOOH$$

VI III V I

(I is reconverted into anhydride etc.).

The third mechanism (scheme III) involves the formation of the α-chlorovinyl ether or α,α-dichloro ether which substances easily convert acylamino acids into their acyl chlorides. These, in turn, are the reactive intermediates in the peptide formation.

Scheme III:

$$HC{\equiv}C\text{-}OC_2H_5 \xrightarrow{HCl} H_2C{=}CCl\text{-}OC_2H_5 \xrightarrow{HCl} CH_3\text{-}CCl_2\text{-}OC_2H_5$$

$$Ac\text{-}NHCHRCOOH + CH_2{=}CCl\text{-}OC_2H_5 \longrightarrow Ac\text{-}NHCHRCOCl + CH_3COOC_2H_5$$

(or $CH_3\text{-}CCl_2\text{-}OC_2H_5$)

$$Ac\text{-}NHCHRCOCl + HCl.NH_2CHR'COOR'' \longrightarrow AcNHCHRCO\text{-}NHCHR'COOR'' + 2\ HCl$$

The question whether racemisation occurs during the synthesis of peptides by means of ethoxyethyne was studied in the synthesis of Cbo-gly-L. phe-gly Et and Cbo-gly-L.leu-gly Me.

By performing the condensation of Cbo-gly-L. phe and glycine ethyl ester-hydrochloride with ethoxyethyne in boiling ethylacetate (method B), we obtained a product, which was considerably racemised (45% of DL-isomer present). This also applied to the synthesis of Cbo-gly-L. leu-gly Me from Cbo-gly-L. leu and gly Me. HCl. If, however, these two syntheses were carried out with the free amino acid ester instead of the hydrochloride, practically no DL-isomer was obtained. Furthermore no racemisation occurs during the synthesis of Cbo-gly-L. phe-gly Et from Cbo-gly and L. phe-gly Et. HBr.

We recommend method C as the standard method for the synthesis of higher peptides from a COOH-component, which itself is a peptide with an optically active C-terminal amino acid. On account of its simplicity method B will be the standard method for dipeptide syntheses or for the extension of a peptide at its NH_2-end with one amino acid at a time. By choosing the proper method of working significant racemisation will generally be prevented in peptide syntheses with ethoxyethyne.